STERILIZATION OF SPACE PROBES

by
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PREFACE

NASA and JPL advise the use of dry heat, ethylene oxide gas, and gamma-radiation for sterilizing space vehicles with any probability of impacting the moon or planets (7,10,15). Dr. George Hobby, in charge of sterilization at JPL, has repeatedly urged that space probes subsequent to RANGER be designed from inception with cognizance of the restraints imposed by sterilization. He has further stressed that heat sterilization (125°C for 24 hours) be employed wherever possible (10).

Kinds of Organisms Likely to Contaminate Space Probes

Assuming that rats and mice, cockroaches and other animals would be visibly apparent, the contaminants likely to escape notice and to which attention must be focused are of microscopic size. In liquids, dust or soil in crevices, microscopic plants (algae, fungi and bacteria) and microscopic animals (chiefly protozoa) may be found. On "clean" metal, plastic or glass surfaces, bacterial cells and spores and fungal spores are almost always present unless special precautions are taken to remove them. Because of their small mass, bacterial cells and especially bacterial and fungal spores (hardy, dehydrated, "resting" cells) are carried in the air. These are the most widespread microorganisms and, because of their dehydrated state and minimal metabolic activity, the most difficult to kill or remove. Such spores and even bacterial cells are found even at high altitudes and ocean deeps. They can be expected to grow and produce more of their kind as soon as they reach favorable conditions (moisture, suitable temperatures).

I. TECHNIQUES FOR STERILIZATION

A. JPL Recommended Methods

A.1 Dry Heat

The most widely used temperature for dry heat sterilization in hospitals is 160°C for 1-2 hours (12). This time applies to the period a given load of supplies is at 160°C. No correction for the time required to bring the load up to temperature is included and would depend upon the size of the load, its heat capacity and heat conductivity. Perkins (12) recommends the following time-temperature ratios for dry heat sterilization.

TABLE 1

Temperature	Time
170°C (340°F)	60 minutes
160°C (320°F)	120 minutes
150°C (300°F)	150 minutes
140°C (285°F)	180 minutes
121°C (250°F)	Overnight

These figures are based on survival times of the more resistant bacterial spores.

The killing action of dry heat for microorganisms depends upon their environment. Organic matter, such as a grease or oil films, thermally insulates entrapped microorganisms such that greased surgical instruments, for example, are sterilized 4 hours at 160°C instead of the usual 1-2 hours.

Sterilization of anhydrous oils and greases thus presents a special problem. Perkins (12) gives a figure of 160 minutes survival of bacterial spores in anhydrous oil, while as little as 20 minutes at this temperature (160°C) suffices if water is present to the extent of 0.5%. Clearly the JPL figure of 125°C for 24 hours is minimal for anhydrous oils. Higher temperatures (say 140°C for

24 hours or 170°C for 4 hours) are recommended. Special consideration should be given any structure containing thermal insulation to allow for adequate sterilizing internal temperatures.

The chief advantages of dry heat as a sterilant is that it kills internally trapped microorganisms. Of the other sterilants considered, only γ-radiation shares this property. Of the two methods, dry heat is much more convenient. Materials particularly suited to dry heat sterilization are metals, glass, air, oils, greases, and thermo-stable plastics. Polyethylene, rubber, germanium transistors and most electrolytic capacitors now in use are heat sensitive and should be avoided where heat is to be used. Dr. Hobby of JPL estimates that at present about 80% of the components of current space probes can be heat sterilized. He further recommends that this method be used as much as possible in the future.

TABLE 2

Times Required for Dry Heat Sterilization of Hospital Supplies
From Perkins (12), 160°C Throughout.

Material	Time	Quantity and Preparation
Glassware	60 minutes	Clean and grease-free
Metal instruments (cutting edge)	60 minutes	Clean and grease-free in metal tray
Petroleum jelly	120 minutes	1/4" layer in glass dish
Powders	120 minutes	1/4" layer in glass dish

In light of the above and other data, the following procedures should be considered when using dry heat for sterilization.

1) sterilize in metal containers (high heat conductivity)
2) spread anhydrous oils and greases into thin films before sterilizing
3) use mechanical convection types of hot air sterilizing chambers. (Perkins recommends electrically operated sterilizers for use in hospitals instead of gas-fired sterilizers largely because of

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greater precision in temperature control).

4) keep all glass, plastic and metal surfaces free of oil and grease, or else allow extra time or higher temperatures for sterilization.

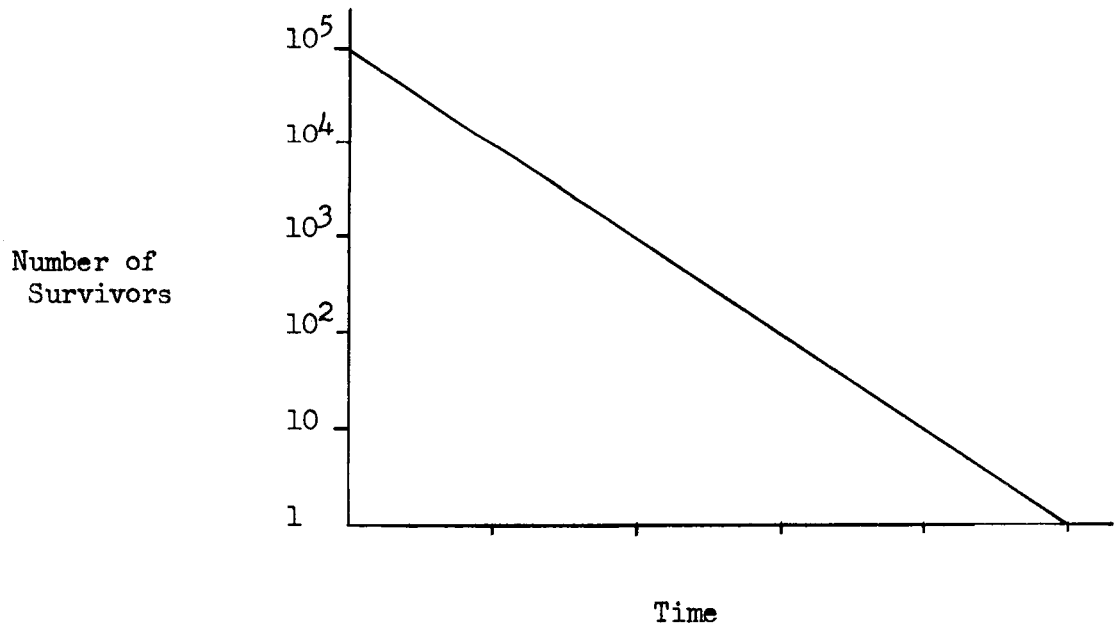
5) use thermistor probes in least heat-accessible areas of the packed sterilizer to monitor temperature.

6) use SPORDEX technique (see Section on sterilization efficiency) with sterilized load to insure that sterilization has taken place.

In general, survival of microorganisms at high temperature vs. time of exposure is logarithmic. At least this is a good working hypothesis. The term "decimal reduction time" (D), defined as the time to reduce the number of survivors 10-fold, is a useful index of the resistance of microorganisms to heat. A hypothetical graph of survival versus time at a given lethal temperature is given below.

FIGURE 1

Hypothetical Thermal Death Time Graph



The thermal death curve, determined empirically for a given organism at a given temperature, can be described by the equation

$$K = \frac{1}{t} \log \frac{N_o}{N_t}$$

where K is a rate constant ($= \frac{1}{D}$) with dimension minutes^{-1}, t is in minutes, N_o is the initial number of organisms and N_t the number remaining at time t. D values are available for moist heat sterilized canned food stuffs but, unfortunately, not for dry heat sterilization of gyros, amplifiers, and the like. Until such data are available we should use 125°C for 24 hours as a minimum time-temperature ratio.

Parker (11) has reviewed the sterilization of air by heating. Heating air at 425°F for 24 seconds allows a probability of survival of bacterial spores of only 10^{-6}. For each 50° rise in temperature the exposure period is roughly halved. At 575° only 3 seconds are required. Air can also be sterilized by filtration, as discussed later.

A.2 Ethylene Oxide (EtO)

Ethylene oxide is a colorless gas at room temperatures. It liquifies at 10.8°C and freezes at -111.3°C. Its density is 0.882 ($d \frac{10}{10}$). It is soluble in water, alcohol and ether. Concentrations of 3% or greater in air support combustion and explosion. In this respect it is similar to ethylether. However, a 10% mixture in CO_2 is not flammable when mixed with any proportion with air. Higher concentrations of EtO (12%) in Freon-12 or 11% EtO in an equal mixture of Freon-12 and Freon-11 are likewise non-flammable in all mixtures with air (5). These mixtures of EtO and Freon are obtained from the Matheson Company.

EtO is a universal disinfectant killing bacteria, fungi, viruses, and other organisms effectively (13,14). Bacterial cells are only about 10 times as

sensitive as bacterial spores. (This is also true for β-propiolactone). With other sterilants the cells are about 1000 times as sensitive as the spores).

As a gaseous sterilant EtO has two distinct advantages:

(1) it is extremely penetrating (materials wrapped in muslin, paper, or polyethylene film are readily sterilized), and

(2) it is effective at low relative humidity (20-40% RH is optimal).

The effective penetration is also a disadvantage in that sterilizing chambers must be tightly sealed to prevent leakage of EtO vapor. If its concentration falls due to leakage, more gas must be added if sterilization is to be effective (8, 14).

Rubber, some plastics and leather sorb EtO, reversibly; oils and organic compounds may react chemically with EtO. Objects made of these materials require several hours air-wash before they can be safely handled. Sorbed EtO or EtO solutions in water cause skin burns in concentrations as low as 1%. EtO vapors cause nose and eye irritation, loss of equilibrium, dyspnea and death. A maximum allowable concentration for people is 100 ppm in air; 3000 ppm may be tolerated for 60 minutes; 50,000 to 100,000 ppm are fatal in minutes (8). Obviously care must be exercised in handling EtO in closed spaces. In sterilizing chambers designed for human entry EtO concentrations may be monitored with a gas chromatograph to insure that entry is not attempted until the EtO concentration is reduced to a safe level. A Freon leak detector would also be desirable.

EtO - Freon mixtures (warmed to 55°C to insure the vapor state) are used at room temperature and pressure to sterilize gloved dry-boxes and "germ-free" hoods (1). Exposures of 6-8 hours to EtO are effective in sterilizing surfaces of contents of the boxes, after which sterile air is flushed through the box (the air is first passed through a bacterial filter) for about 12 hours to remove the EtO.

Collapsing the boxes (they are usually made of flexible plastic)(1) before gassing with EtO insures maximum EtO concentration and minimum dilution with air in the box.

Shorter exposures to EtO are effective in as little as two hours if a pressure chamber (18 psi) is used at 130°F, or in 4 hours (5 psi) at 130°F. A vacumm (28 in. Hg) is drawn on the chamber before injection of EtO gas to avoid dilution with air (8).

For dense materials or for sealed objects such as vacuum tubes, EtO is only a surface sterilant. With this limitation, it may be used on a wide variety of materials and is appropriate for large objects which can be accommodated in sealed chambers. Cylinders of EtO - Freon mixtures could be used at the launch site, even atop the gantry, for sterilizing the exposed surfaces of a probe providing it were sealed within a nose cone or shroud. However, 6-8 hours exposure would be required at atmospheric pressure following which a period for purging with sterile air would be required before launch.

The only materials known to be adversely affected by exposure to EtO vapor are those with which it may undergo chemical reaction: some organics and oils. Those materials which sorb EtO (rubber, plastics and leather) are ready for use after several hours flushing with sterile air. Care should be taken to keep EtO from condensing to liquid drops which might be more corrosive (8, 14).

A.3 Gamma Radiation

Because of the costs, personnel hazards and safety measures attendant to the large amount of radioactive materials required to produce sterilizing doses (10^7 roentgens from Co^{60})(15) radiation should be considered only as a last resort. Apparently few materials can be sterilized with gamma-radiation, without adverse

effect, that cannot be sterilized with dry heat. Norair Nuclear Sciences Report NSR 60-3, 17 March 1960, "Effects Of The Radiative Environment On Space Systems" should be consulted for further references.

Materials adversely affected by a dose of 10^7 r include: rubber tires, teflon, transparent materials, seals, resistors, capacitors, hoses and flexible couplings, thermal insulation, lubricants, flotation fluids, textiles, and some lens materials.

Materials not damaged include explosives, transformers, ceramics, plastic electrical insulation (except teflon) and structural metals.

Co^{60} sources available for public use include the following:

TABLE 3

Location	curies Co^{60}	dose rate r/hr	volume of "hot box"
Lockheed Aircraft Palo Alto	1000	2×10^6	0.003 ft^3
Stanford Research Institute Menlo Park	2000	5×10^5	1 ft^3
Cook Electric* Chicago	62,000	5×10^5	20" dia, 14" high
Shell Chemical**	18,000	8×10^6	0.66 ft^2

* the cost in this case for 10^7 r (20 hours exposure) is about $240.00/chamberful

** not available for public use

B. Other Methods of Sterilization

B.1 Moist Heat

Boiling water will kill bacterial cells (but not spores). For this reason moist heat under pressure is more widely used as a sterilant. At 15 psi the required temperature will be 121°C; at 20 psi, 126°C. Only about 15 minutes at 121°C and 15 psi are to kill bacterial cells and spores. Commercial autoclaves usually operate at this temperature and pressure. Very rarely highly resistant spores may survive, however. In spite of this, the autoclave is the most widely used sterilizing device in microbiological laboratories, hospitals, and in canneries (in modified form). Items desired to be kept dry during autoclaving may be wrapped in paper or muslin (not polyethylene), to extend the range of objects sterilizable.

B.2 β-propiolactone. (BPL)(3).

β-propiolactone is a liquid at room temperature. It melts at -33.4°C and boils at 150°C (decomposes). Its density is 1.149 (d_{20}^{20}). It dissolves in water to the extent of 37% (v/v) at 25°C, miscible with alcohol (reaction takes place), acetone, ether and chloroform. In water it hydrolyzes in minutes to give hydracrylic acid (not a disinfectant). Purified β-propiolactone polymerizes in 6-8 weeks during storage at 54°C but keeps for several years at 40°C (6).

Like EtO, β-propiolactone is a universal disinfectant (13). Bacterial spores are only less sensitive than bacterial cells by a factor of about 4.

As a disinfectant BPL may be used as a 1% solution in water or methanol (it is apparently lethal to spores in minutes in this form) or as a vapor. Unlike EtO, BPL vapor requires a high humidity (70% RH, or greater) to be effective. It is not very penetrating and may be used in ordinary rooms if cracks are sealed with

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masking tape (16). It is effective only as a surface sterilant.

Increasing the concentration of BPL vapor in air decreases the time required for sterilization. For example, spores of the bacterium Bacillus Subtlis (globigii) are killed to the extent of 99%:

TABLE 4

in 120 minutes with BPL concentration of 0.1 mg/liter air,
65 minutes at 0.2 mg/liter air,
30 minutes at 0.4 mg/liter air,
10 minutes at 0.6 mg/liter air, (from Reference 6)

Concentrations of 2% or greater in air are flammable. This concentration is unlikely to be reached, even accidentally, since air (at 30°C) is saturated with BPL vapor at about 0.6% (6). (It is not necessary to elevate room temperature for BPL to be an effective sterilant).

BPL will apparently replace formaldehyde as a disinfectant for large areas. It leaves no residue and is non-corrosive. In practice BPL is atomized (17) (in as fine a mist as possible) into a room using one gallon of 97% BPL for each 12,000 to 16,000 cubic feet of room volume (16). Care must be taken to avoid condensation of liquid droplets as these may craze varnishes and leave a residue. Water vapor is injected to keep the relative humidity 70% or greater. Two hours exposure effectively kills microorganisms and their spores.

BPL is toxic to humans. It is an effective lachrymator and irritant. Thus, if you can enter the room without discomfort, the BPL concentration is below the toxic level (0.1 mg/liter air)(16). Extended contact with skin is carcinogenic and causes erythema and vesication in rats. If necessary, rooms containing BPL

may be entered if a gas mask with charcoal cannister is worn with a gas impermeable suit. At a BPL concentration of 0.05 mg/liter air only a slightly sweetish odor is noticed. Following the two hour BPL exposure the room should be flushed with several changes of sterile air to insure reduction of BPL concentration to a safe working level (16).

B.3 Other Disinfectants

Most chemical disinfectants in use now lack the broad spectrum of action of EtO and BPL. Formaldehyde vapor was used earlier to disinfect large areas. It leaves a polymerized residue, has poor penetration, requires high humidity and the odor is difficult to get rid of. It will be replaced by β-propiolactone (13).

Compounds of mercury are not very effective in killing bacterial spores. Arsenates are not effective against fungi. Compounds of silver, copper, quaternary ammonium compounds, and phenolics (such as hexachlorophene) have been used in agriculture or in the pharmaceutical industry, but none of these are universal disinfectants and their use would not be justified in this application.

A number of organic compounds, having a similar mechanism of action to EtO and BPL (alkylating agents) may be useful. Methylbromide has been investigated in some detail but is less effective than EtO or BPL, although it is non-flammable. The Chemical Warfare Group at Camp Detrick, Maryland, have listed chloropicrin, propylene oxide, epichlorohydrin and ethylimine as substances requiring investigation as disinfectants (13).

Chlorine and sulfur dioxide gases, although effective sterilants, are too corrosive to be useful. Further information is required for peracetic acid (2), suggested as a sterilant for use on air-locks of germ-free boxes (1).

Germicidal lamps emitting ultra-violet light are quasi-effective surface sterilant sources but cannot be relied upon to kill spores or dust particles or in crevices. Such lamps should probably be used in large "clean" assembly rooms to help keep down contamination.

B.4 Bacterial Filters

Filters of small pore size (< 1.0 micron) are useful in producing sterile air. Preformed slabs of slag wool, 3 inches thick, and compressed to a density of 17 lbs/cu.ft. and with fiber diameter less than 6 microns have been used, but air velocities must be kept below 0.5 ft/sec. The slag wool should be sterilized with dry heat to prevent channeling. Glass wool filters of comparable fiber diameter are also effective. Flow rate is also limited to less than 0.5 ft/sec. with glass wool filters. Use of pre-filters to remove dust, oil, etc., increases the life of sterilizing filters and should be used wherever possible (11).

Thin cellulose membranes containing uniform pores of suitable diameter for filtering out bacterial cells and spores have been developed by the Millipore Filter Corp. (9), Isopore Filter Co., and others. These have been widely used in laboratory scale sterilization of liquids as well as air. Flow rates of water and air through these filters are given below (9).

TABLE 5

Filter	Pore Size Microns	Flow rate cc/min at 70/cm hg differential pressure at 25°C	
		Water	Air
SM*	5.0	560	70,000
SS*	3.0	400	45,000
RA*	1.2	300	38,000
HA	0.45	80**	9,600
PH	0.30	40	4,200
VC	0.10	3.6	600
VF	0.01	1.1	100

* Pore size too large to retain bacteria.

** Equivalent to 20 gals per minute per sq. ft. at 15 psi.

Millipore filters decompose in air above 125°C, but in oxygen free systems they may be used up to 200°C. The filters dissolve in some liquids (methanol, acetone and other ketones, ether - alcohols, esters, and glacial acetic acid). Most hydrocarbons, chlorinated hydrocarbons, acids, and bases have no effect. Electrostatic charges build up on the filters resulting from friction in the filtration of gases (9) which increases their effectiveness.

The versatility of these filters is surprising. Among the uses of interest here are: sterilization of air and liquids (possibly even cryogenic fuels), including coolants, damping fluids and lubricants, and in quality control checks for sterility of gasses and liquids.

The largest filter advertized is 293 mm diameter which is stated to operate from -380°F to +275°F (or +350°F if oxygen is excluded), and with a maximum pressure differential of 60 psi. Flow rates for pre-filters and sterilizing filters are given below (9).

TABLE 6

Flow rate in gallons per minute at 30 psi differential pressure at 25°C except as noted for 193 mm filters

Material	Pre-filter		Sterilizing Filter	
	3.0 μ pores	0.45 μ pores	0.45 μ pores	0.22 μ pores
Air*	160	85	175	40
Distilled Water	10	6	12	2.5
Trichlorethylene	12	7	—	—
Kerosene (JP-4)	6	3	—	—
Fluorolube 3000/120**	0.4	0.15	—	—

* cubic feet per minute at 15 psi differential pressure
** at 200°F

B.5 Preventive Measures

In sterilization we are dealing with probabilities of killing or removing contaminants. The probability of a given spore surviving at 125°C for 24 hours is less than that at 125°C for 10 minutes but it is nevertheless finite. Likewise, if we start with 10^9 spores the probability of one spore surviving any sterilizing procedure is greater than if only 10^6 spores were initially present. Thus we should attempt to exclude contaminants from working areas, sterilize working areas frequently with β-propiolactone vapor, keep the air as dust-free as possible (since spores may get a free ride on particulate material) and keep all work free of surface grease and as smooth as possible (spores can find a good place to hide in cracks and crevices) even though sterilization will be carried out subsequently. Assembly should be accomplished in such clean, dust-free areas. Assembly and inspection personnel should not enter the area in street clothing. Special clothing, shoes and hair coverings should be provided fresh at each entry.

Measures such as these, while expensive, time consuming, and of high nuisance value will help keep bacterial, fungal and spore populations to low levels and will decrease the probability of sending aloft a contaminated probe just as effectively as will the sterilization procedures themselves.

C. Means of Choice for Sterilizing Particular Materials

General Recommendations:

gases - filtration or dry heat

liquids - filtration, dry heat (oils, greases,)or moist heat (water solutions)

solids - dry heat for interiors and exteriors

EtO or BPL for exteriors only

C.1 Gases

Sterile air for purging EtO and BPL vapors should be obtained by filtration or heat depending upon circumstances. Millipore filtration with the HA (0.45μ) filter will usually be most convenient.

C.2 Liquids

Silicone oils and greases should be best sterilized with dry heat. Freon and, at least ideally, cryogenic liquids (LOX, liquid hydrogen and nitrogen) should probably be sterilized by filtration in the gaseous state in flow systems prior to liquifaction (using sterile compressors, piping and tankage). Filtration of cryogenic liquids might also be considered. However, this point needs further clarification.

Any aqueous solutions are most easily sterilized in batches by autoclaving. Millipore filters (0.45 μ) may be used in flow systems.

C.3 Solids

Structural materials (aluminum, stainless steel, titanium, magnesium) are most easily sterilized with dry heat although radiation could be used. EtO and BPL can be used for surfaces of such materials. Heat used in forming these metals probably kills any embedded microorganisms so that surface sterilization should be satisfactory. This is not true for all plastics. These should be heat

sterilized to kill embedded organisms. Polyethylene, which will not tolerate the heat treatment, is quite permeable to EtO. Polyethylene structures should be thus sterilized. Teflon, nylon plastics used for wiring insulation and epoxy glues should be heat sterilized.

Ceramics and optical glasses should be heat sterilized although EtO and BPL may be used (heat in forming should kill embedded organisms).

Electronic components constitute a special problem in that each one must be considered as a separate case depending upon its constituent materials, method of manufacture and intended reliability. Transistors, non-electrolytic capacitors and resistors externally sterilized with EtO were found to be internally contaminated (15). On the other hand photomultiplier tubes are rendered internally sterile by heat during manufacture.

In general, heat should be considered as the sterilizing agent of choice for electronic components. Dr. George Hobby, JPL, has sterilized an expensive gyro with heat without any apparent malfunction resulting. However, due to the possibility of reduced operating life each component to be used should be individually performance-tested for heat tolerance.

It is possible that some simple components, possibly electrolytic capacitors, for example, will not survive heat sterilization. In this case the alternatives are: (1) sterilize with gamma-radiation; (2) manufacture aseptically; (3) choose a substitute component which can be heat sterilized. It is recommended that these alternatives be tried in reverse order to which they are listed.

II. Packaging and Handling Sterile Materials

A. Packaging

Internally sterile assemblies may be wrapped in thin polyethylene bags (approximately 0.001") and be resterilized with ethylene oxide. For small items a gas sterilizer (1) using EtO at 15 psi and 130°F is quickest. For items too large for the sterilizer EtO can be vaporized in the bag then purged afterwards with sterile air for 6-8 hours. Paper wrappings can also be used in gas sterilization but these are subject to tearing. Likewise, subassemblies can be placed in sealed cans and heat sterilized for shipment to a final assembly area.

B. Handling Sterile Materials

Trained microbiologists skilled in aseptic technique must be responsible for handling all sterile materials. All assembly personnel must be trained by a competent microbiologist in aseptic technique before attempting such work and then only under the supervision of a microbiologist. Aseptic technique is more of an art than a science and considerable practice is required to achieve proficiency in handling sterile materials without contaminating them.

Gloved "dry-boxes" sterilizable with EtO can be used for aseptic assembly of small components. The larger "dry-boxes" are about 2 ft. x 3 ft. x 4 ft. in size (1). Larger assemblies might be taken into dust-free rooms which can be sterilized with EtO or BPL and which are equipped with air locks for entry of internally-sterile components and properly clothed and trained personnel.

A typical procedure might be as follows:

1. Bring all internally-sterile components and tools for assembly into clean room and remove protective packaging.
2. Sterilize room and surfaces of components with BPL or EtO (depending

upon how well the room is sealed and the humidity tolerance of the components) and purge with sterile air.

3. Enter assembly personnel through air lock to assemble components and place assembly in transportable package.
4. Exit assembly personnel.
5. Resterilize package and outer surface of its contents with EtO or BPL and purge with sterile air.
6. Remove package.

Although such a procedure may seem elaborate and time-consuming it is still possible that contamination can occur. Spores brought in by personnel could be sealed between interfaces and not be subjected to the sterilizing gases. BPL solution in an atomizer should be kept handy to spray such interfaces before they are joined. An extra degree of insurance will be obtained by having competent personnel working under supervision of a microbiologist - assembly engineer team.

Here, as in selection of sterilization methods for components, each job may require a different technique or program of techniques.

III. Quality Control Procedures

A. Introduction

One of the biggest problems pertinent to sterilization of space probes is to know when sterilization has been effected, or the reverse, when contaminants are present. Since magnifications of approximately 1,000 are required to see many microorganisms (viruses are not seen except at higher magnifications), visual observation is not effective except in specialized cases. One such case is for liquids and gases (air). The Millipore Filter Corporation has developed field monitors which collect particles on membrane filters (9). The opaque filters are rendered transparent by the immersion oil used with microscope objectives allowing direct counts of particles collected by the filters. Pressurized fuel systems and air streams may be monitored in this way. These techniques can be of great advantage where laboratory facilities are not handy since the filters can be aseptically packaged and returned to the laboratory for analysis; a good way to check on subcontractors.

Detecting contamination of solids presents a greater and more time-consuming problem in that microorganisms must be scraped, washed, or brushed from the solid surfaces and grown up in liquid culture media. Up to seven days, or more, are required for visible growth to occur in some cases. Since the probe cannot usually sit on the gantry for a week while we wait for evidence of contamination, reliable means of sterilization must be checked out <u>in advance</u> and the probability of contamination pre-determined. This will allow us to say that the probability that a given probe is contaminated is some value (less than the 10^{-6} specified)(15). To do this requires stringent checks for contamination at all stages involving sterilization and aseptic handling, and this requires competent personnel trained in microbiology.

B. Recommendations

It is thus recommended that if Norair intends to contract work involving sterilization of space probes, that a microbiology laboratory be set up in the Quality Control Group and that this laboratory be staffed with microbiologists (defined as any graduate in microbiology, bacteriology or in special cases, botany or zoology from an accredited 4-year college). This group should be responsible for the sterilization of space probes including inspection, selection of procedures, training of assembly personnel in aseptic handling and assembly, and quality control for Norair produced and subcontractor produced sterile components. This group should be aware of the present Biostronautics Laboratory, which is concerned with sterilization from a research viewpoint, and the existing opportunity for consultation and cooperation. Members of the group should strive to keep aware of the available means of sterilization since new advances may be expected to keep coming due to the needs of space research, advancing food technology, and pharmaceutical technology.

C. Tests for Contamination and Sterility

C.1 Efficiency of Sterilization

Sterilizing chambers in which temperature, humidity, pressure or gas concentration is critical require indicators, preferably continuous recording, of these variables to insure that a specific load of materials has actually been exposed to standard sterilizing conditions. BPL and peracetic acid solutions should be frequently standardized by chemical analysis to insure effective concentrations. A method for BPL analysis is given by Hoffman and Warshowsky (6) and for peracetic acid by Greenspan and MacKeller (4).

The American Sterilizer Company has developed a series of test strips impregnated with living bacterial spores of high, but known, resistance to

sterilization. The heat requirements and spore population size are well established and are provided with each set of strips. These, when included with a load of materials to be sterilized, give a positive test of sterilizing efficiency. The following SPORDEX strips are available (1).

TABLE 7

Organism	Spore Population	Established Resistance to Dry Heat Survives	Killed
Bacillus subtlis (globigii)	1,000,000	121°C for 30 min.	121°C for 240 min.
Clostriduim sporogenes	200,000	121°C for 60 min.	121°C for 180 min.
Bacillus stearothermo-philus	200,000	121°C for 30 min.	121°C for 120 min.

Data of the American Sterilizer Company

After exposure of the strips to a sterilizing environment they are inoculated into nutrient broth and incubated for seven days. Appearance of turbidity during this period indicates bacterial growth; that the spores were not killed; that sterilization was not effected.

C.2 Tests for Contamination

Because contamination is not likely to be visually apparent on solid surfaces or within plastic structures, tests for contamination will involve inoculating nutrient broth with: (a) the whole object if it is small, (b) parts of broken objects where internal contamination is possible, (c) with sterile water in which the object has been washed, or (d) with a sterile brush which has been brushed over the surface of the object. Other similar tests might be devised. In each case turbidity

developing in the inoculated media will indicate contamination. Since even microbiologists skilled in aseptic technique sometimes accidentally contaminate items they are handling, such tests should probably be carried out with multiple samples.

The same type of test can be performed with liquids and gases by sampling with a membrane filter and aseptically transferring the filter to a flask of nutrient broth.

Since not all organisms will grow in a given nutrient broth several different media should be inoculated for maximum reliability of the tests.

The U.S. Pharmocopeia, 14 ed. (1950) lists a few simple techniques for conducting sterility tests which are considered reliable and should be consulted.

IV. Sterilization of Return Vehicles

Assuming that only surfaces of a probe and its sampling gear and sample storage chamber would possibly become contaminated by extraterrestrial life, the best means of sterilization would probably be with EtO or BPL vapor. The further advantage of these means is that lightweight portable equipment can be used. The equipment would include a storage chamber (pressurized) for EtO or BPL, a triggering mechanism, and a system of tubes to distribute the vapors. Use of BPL would require an atomizer and water vapor injection system, thus an EtO system might require less weight.

The gas would be allowed to flow over potentially contaminated surfaces during flight through free space when convection, tending to blow away the vapor, would be minimal. No purging with air should be necessary since the gas would be blown away on entering the earth's atmosphere.

An alternative method might involve utilizing friction-produced heat on re-entry. Heat might be conducted over potentially contaminated surfaces to effect temperatures required for sterilization in a few minutes. Or a combination of the two approaches could be used: heat conduction of external probe surfaces with EtO gassing or sampling gear and sample storage chamber.

In any case, sample storage chambers should be sealed to prevent opening on impact with the earth since our sterilants might not be effective on extraterrestrial organisms. Such chambers should be returned to a laboratory and opened in a "dry box" (1) as a safety precaution against contaminating the earth surface. The sample storage chamber should be made of material not corroded by sea water in the event the probe lands in the ocean.

Use of EtO to sterilize external surfaces of a returning probe has the advantage that, if the probe is damaged and surfaces are exposed which are not supplied with conducting elements, sterilization of these surfaces would also be effected. 25

V. Restraints Imposed by Sterilization

1. All components subject to internal contamination by organisms should not be adversely affected by heat sterilization (125°C for 24 hours). The alternative sterilant, gamma-radiation is all but excluded because of costs of facility and personnel hazard.

2. Wherever possible, components should be tolerant of high humidity (75% RH) to allow use of β-propiolactone vapor for sterilization of external surfaces. This will simplify the design of sterile assembly rooms in that they will not need to be airtight as required by the ethylene oxide method.

3. All external and internal surfaces should be as smooth as possible to avoid presenting hiding places for microorganisms and to reduce costs of quality control procedures.

4. Costs of sterilization facilities and adequate quality control personnel and a laboratory facility should be reflected in space probe proposals involving sterilization.

5. Facility and personnel for testing reliability of components and systems following dry run heat sterilization should be allowed for in space probe proposals. Use and testing of alternate components should be considered, if necessary, and the effect of substitutions considered in terms of overall reliability.

VI. Areas for Further Study

The effects of heat sterilization on the long-term reliability of non-structural components seems to be the largest area of mystery and one in which we might gather some experimental data. This would lead to increased awareness of the possible restraints imposed by sterilization, familiarity in the aseptic handling of materials, and in engineering of heat sterilizers. Such experience could only work to strengthen Norair capability in the space probe field. There is every reason to believe that the requirement for sterilization of probes will be continued indefinitely, for one reason or another.

Setting up a "familiarization program" in the use of EtO and BPL might also be worthwhile for the reasons listed above. Such effort should include study of design possibilities for sterilization of payloads within nose cones and of return vehicles, as well as experimental laboratory work.

Another area of concern is <u>aseptic assembly</u>. A sterile assembly room, similar to the well known clean rooms for assembly of intricate electronic components, might be set up. Design of air locks, wall coatings and use of sterile clothing, disinfectant soaps, etc., might be undertaken depending upon further evaluation of the need for aseptic assembly. At this date I am not convinced that the need exists or that sterile assembly of large structures (larger than that which can be remotely assembled in a gloved, 2' x 3' x4' dry box) is practical.

GLOSSARY

Alkylating Agent..... A chemical which reacts with a cyclic compound by substituting an alkyl radical (general formula $C_n H_{2n+1}$) for a hydrogen atom of the cyclic compound.

Aseptic Technique..... Means of handling materials excluding microorganisms.

Autoclave..... A device for sterilizing objects by steam heat at high pressure.

Carcinogen..... A cancer producing agent.

Disinfectant..... An agent which destroys or inhibits microorganisms causing disease.

Dyspnea..... Labored breathing.

Erythema..... Redness of the skin in patches of variable size and shape.

Lachrymator..... A substance which causes undue secretion of tears; a tear gas.

Microorganism..... An organism (living thing) of microscopic size; may be plant or animal; especially bacteria, protozoa, fungi, virus, richettsia, some algae.

Spore..... A reproductive cell, usually with a thick wall and low water content enabling it to survive in adverse environments.

Sterilant..... An agent which kills or removes all forms of life.

Sterilization..... Removal or destruction of all forms of life.

Thermal death time..... Time required to kill an organism with heat at a specific temperature.

BIBLIOGRAPHY

1. American Sterilizer Co., Erie, Pa.
2. Becco Chemical Div., Food Machinery and Chemical Corp., Buffalo 7, N.Y.
3. Celanese Corp. of America, New York, N.Y. supply 95% commercial grade and 99% purified grade β -propiolactone.
4. Greenspan, F. P., and D. G. MacKeller, 1948, Anal. Chem. 20, 1061.
5. Haenni, E. O. W. A. Affens, H. G. Lento, A. H. Yeomans and R. A. Fulton, 1959 Ind. & Engn. Chem. 51, 685. New nonflammable formulations for sterilizing sensitive materials.
6. Hoffman, R. K., and B. Warshowsky, 1958, Appl. Microbiol. 6, 385. Beta-propiolactone vapor as a disinfectant.
7. Jacobson, N. F., 1961, The operations problem of sterilization. Paper presented to the Amer. Rocket So., Missile and Space Testing Conf., Los Angeles.
8. Lloyd, R. G., and E. L. Thompson, 1958, Gaseous sterilization with ethylene oxide. Amer. Sterilizer Co., Erie, Pa.
9. Millipore Filter Corp., Bedford, Mass. Technical Brochure No. 759.
10. Missiles and Rockets, Mar. 20, 1961. How RANGER payload is sterilized pp. 27-28.
11. Parker, H., 1958, Sterilization of equipment, air and media. In: Biochemical Engineering (R. Steel, ed.). Heywood & Co., Ltd., London. pp. 95-121.
12. Perkins, J. J., 1954, Bacteriological and surgical sterilization by heat. In: Antiseptics, disinfectants, fungicides and sterilization (G. F. Reddish, ed). Lea & Febiger, Phila., Pa. pp 655-719.
13. Phillips, C. R., and B. Warshowsky, 1958, Ann. Rev. Microbiol 12, 525-50, Chemical disinfectants.
14. Phillips, C. R., 1954, Gaseous sterilization: In: Antiseptics, Disinfectants, Funcicides and Sterilization (G. F. Reddish, ed.). Lea & Febiger, Phila., Pa.

15. Posner, J. (ed.), 1960, Proceedings of meeting on problems and techniques associated with decontamination and sterilization of spacecraft. NASA Technical Note No. D-771.

16. Spiner, D. R., and Hoffman, R. K., 1960, Appl. Microbiol. 8, 152-55. Method for disinfecting large enclosures with β-propiolactone vapor.

17. Atomizers are available from Combustion Equipment Div., Todd Shipyards Corp., Elmhurst, Long Island, N.Y. and from Tokheim, Fort Wayne, Ind.